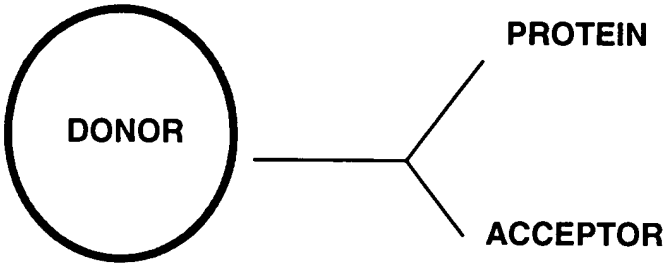
MTP Activity Kit

Microsomal [triglyceride] Transfer Protein Activity Kit

Overview - The microsomal transfer protein (MTP) is a membrane bound protein present in normal human liver cells. The MTP is responsible for the assembly and secretion of very low density lipoprotein (VLDL). The MTP Activity Kit includes donor and acceptor particles. Incubation of donor and acceptor with MTP source results in the MTP mediated transfer of fluorescent neutral lipid. The fluorescent neutral lipid is present in a self-quenched state when contained within the core of the donor. The MTP mediated transfer is observed by the increase in fluorescence intensity as the fluorescent neutral lipid is removed from the self-quenched donor to the acceptor.

FOR RESEARCH USE ONLY

COMBINE:

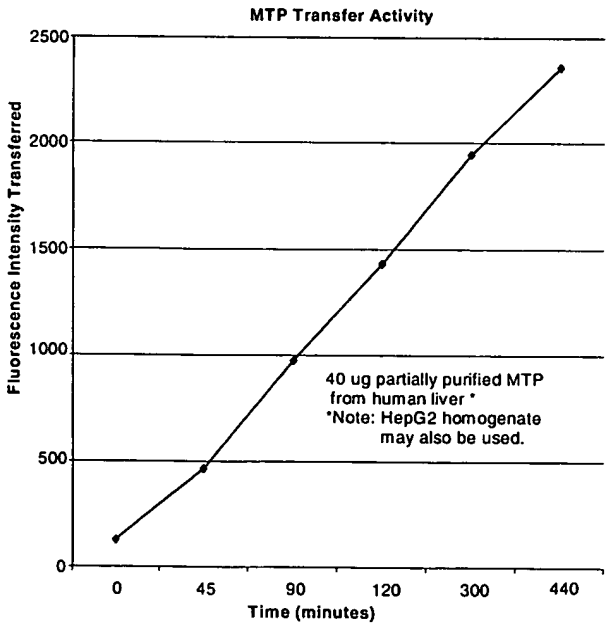


INCUBATE: **37°**

READ: **Activity is Assessed as an Increase in Fluorescence**

METHOD

- Combine 10 µl donor and 10 µl acceptor with MTP source in 0.5 ml total volume of buffer (10 mM tris, 150 mM NaCl, 2 mM EDTA) at pH 7.4
- Incubate for 3 to 6 hours at 37 degrees centigrade
- Read assay in a fluorescence spectrometer at excitation wavelength of 465 nm and emission wavelength of 535 nm



Protocol for Preparing HepG2 Cell Homogenate

- HepG2 cells are grown in 75 cm^2 T-flasks until confluent.
- The cells from 6 flasks are suspended in a total of 5 ml of homogenization buffer (approximate protein concentration will be 10 mg/ml).
- The suspension is sonicated on ice with five 5 second bursts in a 550W sonicator fitted with a microtip on power setting 4.
- 100 µg of homogenate protein is used in the assay.

Note that it is not necessary to spin down cell debris to make a low-speed supernatant, nor is it necessary to partially purify membranes to assay MTP activity.

Homogenization Buffer: To 100 ml of 10mM Tris/150 mM NaCl/ 1mM EDTA, pH 7.4 add 0.5 ml of 100 mM PMSF (Sigma P-7626) in ethanol and 2 ml of 1mg/ml leupeptin (Sigma L-3402) in tris-saline.

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NOTES: 50 mM tris interferes with the assay. Fluorescent assays are highly sensitive and will respond to slight changes in assay volume -- **BE SURE TO CAP TUBES** -- microplate incubations must be placed in sealed container with standing water to prevent evaporation. Results from duplicates should be tight. Variability indicates evaporation, inaccurate pipetting or incomplete mixing of assay components.

Donor and Acceptor may be mixed with the buffer and pipetted as one step.

Microplate incubations must be able to raise temperature of assay to 37°C rapidly. Test your incubator with a small container of water and a thermometer. Large, humidified air incubators have caused problems by slowly increasing temperature from 25° to only 34° after three hours. Tubes should be incubated in a water bath type incubator.

Microplates must be compatible with fluorescent assays. Some clear plates contain fluorescent plastic.

The filters must be within specification. An excitation filter of 485 nm with a 20 nm bandwidth may **NOT** be used. This filter will incompletely excite the label and the standard curve will appear to work but your protein activity results will be low.

RECOMMENDED PLATES

- Millipore CytoPlate low fluorescence plastic #CFCP N96
- Dynex Laboratories black plates (must be used only with top reading plate readers)

Ordering Information

#RB-MTP	MTP Activity Kit – 100 assays
#RB-HLV	Partially purified human MTP – 0.5 ml at 20 mg/ml

Contact Information

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Standardization

The batch number of the kit and concentration of the fluorescent substrate is printed on the label.

The standard curve to derive the relationship between fluorescence intensity and mass transfer is generated by dispersing donor particle in isopropanol. Spectrally pure (HPLC grade or better) isopropanol is utilized as the solvent. There should be no background fluorescence when isopropanol alone is read at EX 465 nm / EM 535 nm.

Prepare six test tubes labeled from 'T0' to 'T5' each containing 1 ml isopropanol, add an additional 1 ml of isopropanol to 'T5'.

Pipette 5 µl donor particle to the test tube labeled 'T5', thoroughly mix (vortex) to adequately disperse the donor particle in the isopropanol.

Transfer 1 ml 'T5' (concentration = (X moles/ml x (0.005)/2) / ml isopropanol) to the test tube labeled 'T4'. Mix and pipette 1 ml from tube 'T4' to tube 'T3', vortex tube 'T3'. Pipette 1 ml from tube 'T3' to tube 'T2', vortex tube 'T2'. Pipette 1 ml from tube 'T2' to tube 'T1', vortex.

Read the fluorescence intensity (EX 465 / EM 535) of the samples from tubes 'T0' to 'T5'.

Apply the fluorescence intensity values of the standard curve directly to your results to express specific activity of the protein.